☐ 1: Blood. 1994 Jun 1;83(11):3279-88. Related Articles, Links

Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines.

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Idiotypic determinants on neoplastic B cells could provide tumor antigens for vaccination of patients with B-cell tumors. Because this approach requires an individual vaccine for each patient, simple methods for obtaining idiotypic antigen are desirable. Using polymerase chain reaction (PCR) with family-based V-gene and J-region primers, the variable region genes of heavy and light chains (VH and VL) of Ig have been obtained from biopsy material from 13 patients with B-cell tumors. In each case, analysis of random clones derived from the PCR product showed repeated, clonally-related sequences, whereas normal lymphoid tissue generated no repeated sequences. In 3/3 cases, the repeated sequences were found to be the same as those in a tumor-derived hybridoma. Mutational patterns in the V-genes differed among the tumors, with follicular lymphoma tending to be more highly mutated. The individual VH and VL sequences have been assembled with a flexible linker sequence to encode single-chain Fv (scFv). The scFv sequences can be cloned into bacterial expression vectors to produce protein, or into vectors suitable for direct vaccination using naked DNA. In a model system, expressed scFv protein retained all idiotypic determinants defined by a panel of five anti-idiotypic monoclonal antibodies (MoAbs). Similarly, expressed scFv proteins from two patients were shown to react with anti-idiotypic antibodies. This approach allows production of potential vaccines from surgical biopsies within 2 to 3 weeks.

PMID: 8193363 [PubMed - indexed for MEDLINE]

☐ 1: Ann N Y Acad Sci. 1995 Nov 27;772:212-26. Related Articles, Links

A genetic approach to idiotypic vaccination for B cell lymphoma.

Stevenson FK, Zhu D, King CA, Ashworth LJ, Kumar S, Thompsett A, Hawkins RE.

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Idiotypic immunoglobulin expressed by a B cell tumor presents a clear tumor antigen which could be attacked by vaccination of the host. Vaccination with idiotypic protein has been shown to induce protective immunity against lymphoma, but application to patients is limited by the requirement of "personal" vaccines for each patient. A genetic approach enables V-region sequences encoding idiotypic antigen to be rescued from tumor biopsies, and to be assembled as scFv fragments. These can be expressed in bacteria to produce recombinant protein, or used directly as naked DNA vaccines. Intramuscular injection of idiotypic DNA from a mouse B cell lymphoma induces low levels of syngeneic anti-idiotypic antibody in serum. Response can be stimulated by co-injection of DNA plasmids encoding either IL-2 or GM-CSF, and T cells which proliferate in response to idiotypic IgM are generated. However, protection against tumor appears to be blocked by continuing secretion of idiotypic antigen from the persisting vaccine vector, which forms immune complexes with serum antibody. Methods for regulating the level of scFv to engage the immune system, but not to block the effector arm are being investigated. Similar control will be applicable to the cytokine vectors, which can deliver encoded cytokines designed to activate immune pathways for tumor destruction. Experience gained in lymphoma may be extended to other tumors with defined tumor antigens.

Publication Types:
- Review
- Review, Tutorial

PMID: 8546396 [PubMed - indexed for MEDLINE]

☐ 1: Protein Eng. 1994 Aug;7(8):1017-26. Related Articles, Links

Multivalent Fvs: characterization of single-chain Fv oligomers and preparation of a bispecific Fv.

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Single-chain Fv proteins are known to aggregate and form multimeric species. We report here that these molecules represent a new class of molecular assembly, which we have termed multivalent Fvs. Each binding site in a multivalent Fv comprises the variable light-chain (VL) domain from a single-chain Fv, and the variable heavy-chain (VH) domain from a second single-chain Fv. Each single-chain Fv in a multivalent Fv is part of two binding sites. We have characterized the multivalent forms of the 4-4-20, CC49 and B6.2 sFvs. The degree of multivalent Fv formation is linker-dependent. Multivalent Fvs cannot form in the absence of an intact linker. Multivalent Fvs can be stabilized by their antigen. The conversion between different forms of the multivalent Fvs can be catalyzed by disassociating agents such as 0.5 M guanidine hydrochloride with 20% ethanol. Multivalent Fvs have significantly different stabilities depending on the specific variable domains from which they are constructed. Two models have been proposed for the structure of a multivalent Fv. We have tested each model by attempting to produce a heterodimer from the anti-fluorescein 4-4-20 and anti-tumor CC49 variable regions. We successfully produced a 4-4-20/CC49 heterodimer that comprises two mixed sFvs. The first mixed sFv is composed of the 4-4-20 VL domain, a 12 residue linker and the CC49 Vh domain. The second mixed sFv is composed of a CC49 VL domain, a 12 residue linker and the 4-4-20 VH domain. The 4-4-20/CC49 heterodimer bound both fluorescein and the tumor-associated glycoprotein-72 antigen. These results support a VH/VL 'rearrangement' model in which each variable domain of a multivalent Fv binding site comes from a different polypeptide chain.

PMID: 7809028 [PubMed - indexed for MEDLINE]

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